PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
WOB 99 AN CEU NEUR		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 08223	23/08/2000	27/08/1999
Applicant		
EC (EUROPEAN COMMUNITY)		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
T/EP 00/08223

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/85 C12N5/10 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GUENAL I ET AL: "Bcl-2 and Hsp27 act at different levels to suppress programmed cell death" ONCOGENE, vol. 15, no. 3, 17 July 1997 (1997-07-17), pages 347-360, XP000876792 figures 1-10 ---	1-11
X	SELIVANOVA G ET AL: "Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain" NAT MED, vol. 3, no. 6, June 1997 (1997-06), pages 632-638, XP002130736 figure 3 --- -/--	1-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 February 2001	23/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lonnoy, O

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CHEN X ET AL: "p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells" GENES & DEVELOPMENT, vol. 10, no. 19, 1 October 1996 (1996-10-01), pages 2438-2451, XP000876795 figures 1-5,8	1-11
X	LIU H ET AL: "Lac/Tet-inducible system functions in mammalian cell lines" BIOTECHNIQUES, vol. 24, no. 4, April 1998 (1998-04), pages 624-632, XP002130737 figure 3	6,8,9
Y		1-5,7, 10-12
Y	SATOH T ET AL: "Free radical-independent protection by nerve growth factor and Bcl-2 of PC12 cells from hydrogen peroxide-triggered apoptosis" J. BIOCHEM., vol. 120, no. 3, September 1996 (1996-09), pages 540-546, XP002130738 cited in the application figures 1,6	1-5,7, 10-12
A	LEIST M ET AL: "Apoptosis, excitotoxicity, and neuropathology" EXP. CELL RES., vol. 239, no. 2, 15 March 1998 (1998-03-15), pages 183-201, XP000876804 the whole document	1-5,7, 10-12
A	WO 96 35124 A (UNIV JEFFERSON) 7 November 1996 (1996-11-07)	
A	WO 95 05738 A (MASSACHUSETTS INST TECHNOLOGY) 2 March 1995 (1995-03-02)	
A	WO 97 24458 A (FOX CHASE CANCER CENTER) 10 July 1997 (1997-07-10)	
A	WO 94 26889 A (IST NAZ STUD CURA DEI TUMORI ;GRECO ANGELA (IT); PIEROTTI MARCO A) 24 November 1994 (1994-11-24)	

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INTERNATIONAL SEARCH REPORT

International Application No
[redacted]T/EP 00/08223

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A (N)	HAVIV R ET AL: "The intracellular domain of p55 tumor necrosis factor receptor induces apoptosis which requires different caspases in naive and neuronal PC12 cells" J NEUROSCI RES, vol. 52, no. 4, 15 May 1998 (1998-05-15), pages 380-389, XP000879428	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-11 (all totally)

Use of cells genetically transformed with a nucleotide sequence coding for the p53 protein or fragment thereof being able to induce apoptosis for toxicity detection; corresponding cells, processes and kits

2. Claims: 1-11 (all totally)

Use of cells genetically transformed with a nucleotide sequence coding for the bcl-2 protein or fragment thereof being able to inhibit apoptosis for toxicity detection; corresponding cells, processes and kits

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/08223

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
[redacted]/EP 00/08223

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9635124	A	07-11-1996	US	5695944 A	09-12-1997
			US	6130201 A	10-10-2000
WO 9505738	A	02-03-1995	NONE		
WO 9724458	A	10-07-1997	US	5741646 A	21-04-1998
WO 9426889	A	24-11-1994	IT	1264447 B	23-09-1996
			AT	150790 T	15-04-1997
			AU	689778 B	09-04-1998
			AU	6926994 A	12-12-1994
			CA	2162677 A	24-11-1994
			DE	69402313 D	30-04-1997
			DE	69402313 T	21-08-1997
			DK	698096 T	27-10-1997
			EP	0698096 A	28-02-1996
			ES	2101537 T	01-07-1997
			FI	955447 A	13-11-1995
			GR	3023878 T	30-09-1997
			JP	8510119 T	29-10-1996
			NO	954556 A	12-01-1996
			NZ	267086 A	29-01-1997

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 99 40 2139

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	GUENAL I ET AL: "Bcl-2 and Hsp27 act at different levels to suppress programmed cell death" ONCOGENE, vol. 15, no. 3, 17 July 1997 (1997-07-17), pages 347-360, XP000876792 * figures 1-10 *	1-11	C12N15/85 C12N5/10 G01N33/50
X	SELIVANOVA G ET AL: "Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain" NAT MED, vol. 3, no. 6, June 1997 (1997-06), pages 632-638, XP002130736 * figure 3 *	1-11	
X	CHEN X ET AL : "p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells" GENES & DEVELOPMENT, vol. 10, no. 19, 1 October 1996 (1996-10-01), pages 2438-2451, XP000876795 * figures 1-5,8 *	1-11	TECHNICAL FIELDS SEARCHED (Int.Cl.7) C12N G01N
X	LIU H ET AL: "Lac/Tet-inducible system functions in mammalian cell lines" BIOTECHNIQUES, vol. 24, no. 4, April 1998 (1998-04), pages 624-632, XP002130737	6,8,9	
Y	* figure 3 *	1-5,7, 10-12	

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	17 February 2000	Lonnoy, O

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 99 40 2139

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
D,Y	SATOH T ET AL: "Free radical-independent protection by nerve growth factor and Bcl-2 of PC12 cells from hydrogen peroxide-triggered apoptosis" J. BIOCHEM., vol. 120, no. 3, September 1996 (1996-09), pages 540-546, XP002130738 * figures 1,6 *	1-5,7, 10-12	
A	LEIST M ET AL: "Apoptosis, excitotoxicity, and neuropathology" EXP. CELL RES., vol. 239, no. 2, 15 March 1998 (1998-03-15), pages 183-201, XP000876804	1-5,7, 10-12	
A	WO 96 35124 A (UNIV JEFFERSON) 7 November 1996 (1996-11-07)		
A	WO 95 05738 A (MASSACHUSETTS INST TECHNOLOGY) 2 March 1995 (1995-03-02)		TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	WO 97 24458 A (FOX CHASE CANCER CENTER) 10 July 1997 (1997-07-10)		
A	WO 94 26889 A (IST NAZ STUD CURA DEI TUMORI ;GRECO ANGELA (IT); PIEROTTI MARCO A) 24 November 1994 (1994-11-24)		

1 The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	17 February 2000	Lonnoy, O

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 99 40 2139

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

17-02-2000

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9635124 A	07-11-1996	US 5695944 A	09-12-1997
WO 9505738 A	02-03-1995	NONE	
WO 9724458 A	10-07-1997	US 5741646 A	21-04-1998
WO 9426889 A	24-11-1994	IT 1264447 B	23-09-1996
		AT 150790 T	15-04-1997
		AU 689778 B	09-04-1998
		AU 6926994 A	12-12-1994
		CA 2162677 A	24-11-1994
		DE 69402313 D	30-04-1997
		DE 69402313 T	21-08-1997
		DK 698096 T	27-10-1997
		EP 0698096 A	28-02-1996
		ES 2101537 T	01-07-1997
		FI 955447 A	13-11-1995
		GR 3023878 T	30-09-1997
		JP 8510119 T	29-10-1996
		NO 954556 A	12-01-1996
		NZ 267086 A	29-01-1997

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
WOB 99 AN CEU NEUR		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08223	23/08/2000	27/08/1999
International Patent Classification (IPC) or national classification and IPC		
C12N15/85		
Applicant		
EC (EUROPEAN COMMUNITY) et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/01/2001	20.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Roscoe, R Telephone No. +49 89 2399 2554

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/08223

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-22	as originally filed				

Claims, No.:

1-11	as received on	05/11/2001	with letter of	05/11/2001	

Drawings, sheets:

1/4-4/4	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5, 9-11
	No:	Claims	1-4, 6-8
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-11

Industrial applicability (IA) Yes: Claims 1-11
No: Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

IV. Lack of Unity

Since applicant has paid an additional examination fee, both invention groups have been examined.

The two inventions, both covering claims 1-12(in part), relate to cells transformed with I: construct expressing p53 and II: transformed with construct expressing bcl-2 and uses thereof to assay toxicity. Bcl-2 (XP000876792) has already previously been overexpressed in a system to analyse apoptotic mechanisms, the same applies to p53 (XP000876795). Further, Bcl-2 and p53 have opposite effects and hence the two claimed systems do not solve the same technical problem.

V. Reasoned statement on Novelty, Inventive Step and Industrial Applicability

The documents mentioned in the present written opinion / International Preliminary Examination Report are numbered as in the search report, i.e. D1 corresponds to the first document of the search report etc.

- **Novelty (Art.33(2) PCT)**

D1 discloses the effect of p53 or of hydrogen peroxide on rat embryo fibroblasts carrying inducible bcl-2 genes. Bcl-2 was shown to slow down at least two stages of apoptosis: decrease of mitochondrial membrane potential and subsequent morphological changes. The bcl-2 gene was cloned downstream of a minimal promoter carrying bacterial tOp sequences. A CMV promoter drives constitutive expression of a tTA fusion protein. In the absence of tetracycline, tTA binds tOp and activates bcl-2 transcription. Addition of tetracycline progressively prevents tTA binding. Cells grown in absence of tetracycline thus express bcl-2 and these cells were challenged with apoptotic inducers and the level of apoptosis determined. Since rat embryo fibroblasts are not neuronal cells, D1 no longer anticipates claims.

D2 discloses a Tet-regulatable His-273 mutant of p53, yet this mutant is not per se capable of inducing apoptosis. Also cotransfected cells with a wild-type p53 expression plasmid (which is assumed to be constitutively expressing p53 since

induction is never mentioned in this context) and fusion peptide 46. Also, tumor cell lines with varying p53 status (wild-type, mutant, null) were treated with peptide 46 and analysed for induction of apoptosis. Nevertheless, active p53 does not appear to have been expressed from inducible system. Hence, not relevant to novelty.

D3 discloses Saos2 (osterosarcoma) and H1299 (small cell lung carcinoma)-derived cell lines having p53 expressed under the control of a tetracycline-regulated promoter. Showed that cells expressing low levels of p53 (levels set by varying level of tetracycline had reduced growth but did not exhibit apoptosis. Further showed that when levels of p53 were low (in tet-regulated cell line which produced lowish levels of p53 even in absence of p53), camptothecin could induce apoptosis in a greater number of cells, despite p53 levels remaining stable. Thus p53 expression could sensibilize cells to induction of apoptosis. It is stated that this situation can be utilized to screen a variety of cancer therapy drugs that may cooperate with p53 to induce apoptosis. Since cells actually employed were not neuronal cells, no basis for novelty objection to present claims.

D4 provides a detailed mechanistic explanation of the Tet system employed (preferably by the applicant). Further, the system is used to express bcl-2 in a NIH/3T3(fibroblast-like)-derived cell line. Further showed that overexpression of Ha-ras from a parallel regulatory system caused apoptosis which could be reduced dramatically by simultaneous overexpression of bcl-2. Since cells actually employed were not neuronal cells, no basis for novelty objection to present claims.

D5 uses a constitutive bcl-2 expressing plasmid to express protein in PC12 cells. Cells were challenged by apoptosis inducers. Not relevant to novelty.

D6 is a review on apoptosis, D7 relates to the regulation of Bcl-2 phosphorylation.

D8 provides a method for evaluating cancer treatments. The p53 gene can be knocked out and the efficacy of the agent to induce apoptosis tested in its absence. D9 disclosure is similar. D10 is irrelevant in present context.

D11 performs various assays on apoptosis in PC12 cells. PC12 is a neuronal cell line which was also used in application. It was shown that bcl-2, which was expressed from a Tet-regulated system, could prevent apoptosis induced by p55-IC. The study is certainly a mechanistic study (compare bottom claim 1). D11 anticipates claims 1-4 and 6-8.

- **Inventive Step (Art.33(3) PCT)**

With respect to the claims in general, it is noted that the functions of bcl-2 and p53 in the context of apoptosis have been recognized and suitable expression systems as used in the above documents are available. Further, it is known that many diseases are associated with the regulation of apoptosis. Hence, the use of the known systems to study such diseases is entirely obvious. D3 even suggests using such a system to screen for anti-cancer drugs. (note: claim 1 refers to neuroblastomas). Obvious that can use system to screen for drugs relating to other disorders associated with apoptosis.

The presently novel claims 5 and 9 are only novel due to involvement of VP16. This is however presently considered an optional and trivial aspect of the regulatory system used.

Claims 10 and 11 are novel since in D11 p55IC is not added to the culture media. It is also arguable whether the process described in D11 can be considered to fall under the kind of screening claimed. Nevertheless, general inventive step objection applies.

- **Industrial Applicability (Art.33(4) PCT)**

The present claims appear to have industrial applicability. Nevertheless it may be better to make it absolutely clear that the cells used in claim 1 cannot be within an animal body.

VIII. **Certain observations**

- **Clarity (Art.6 PCT)**

It is noted that many of the independent claims have features which are optional "such as". These kind of optional features should usually be placed in appended dependent claims.

Claim 11 refers to itself.

CLAIMS

1. Use of cells genetically transformed with the following nucleotide sequences :

- a nucleotide sequence coding for the p53 protein or a fragment or a derived sequence thereof, said fragment or derived sequence being able to induce apoptosis,

- or a nucleotide sequence coding for the bcl-2 protein or a fragment or a derived sequence thereof, said fragment or derived sequence being able to inhibit apoptosis,

- and a nucleotide sequence controlling the level of expression of p53 or bcl-2 proteins, said nucleotide sequence comprising a sequence coding for an activator protein responsible for the expression of p53 or bcl-2 proteins mentioned above either when a specific compound is present in the culture medium, or when a specific compound is absent from the culture medium, the expression p53 or bcl-2 proteins ranging between 0% to 100% depending on the concentration of the specific compound in the culture medium, said specific compound being able to bind to said activator protein thus allowing, or conversely, inhibiting said protein to act as an activator of the promotion of the transcription under the control of which is placed the above-mentioned nucleotide sequence coding for the p53 or bcl-2 protein,

for the detection of developmental or post-developmental toxicity of endogenous or exogenous factors, including drugs and other chemicals, or for the screening of drugs for the treatment of pathologies related to an abnormal inhibition or activation of apoptosis, or for mechanistic studies.

2. Use according to claim 1, characterized in that the genetically transformed cells are chosen among mammalian neuronal cells such as rat PC12 neuronal cell line or any other eucaryotic cells or cell lines.

3. Use according to claim 1 or 2, characterized in that the expression of p53 or bcl-2 proteins is proportional to the quantity of the specific compound added to the culture medium, so that the level of expression of p53 or Bcl-2 in the transformed cells is 0%, or 100%, or comprised between 0% and 100%.

cording to anyone of claims 1 to 3, characterized in that the nucleotide trolling the level of expression of p53 or bcl-2 proteins, is a Tet-Off gene a tetracycline-controllable transactivator (tTA), the tTA comprising a Tet (TetR) linked to a polypeptide which directly or indirectly activates transcription or Bcl-2 proteins, the transcription of the tTA being under the control of an opriate promoter such as a promoter from cytomegalovirus (pCMV).

5. Use according to anyone of claims 1 to 4, characterized in that :

- the polypeptide which directly or indirectly activates transcription of p53 or bcl-2 proteins, is the virion protein 16 from herpes simplex virus (VP16),
- the transcription of the p53 or bcl-2 proteins is under the control of a tTA-responsive promoter comprising a minimal promoter, such as the cytomegalovirus immediate early gene promoter (P_{minCMV}), linked to a tetracycline responsive element (TRE).

6. Cells genetically transformed with the following nucleotide sequences :

- a nucleotide sequence coding for the p53 protein or a fragment or a derived sequence thereof, said fragment or derived sequence being able to induce apoptosis,
- or a nucleotide sequence coding for the bcl-2 protein or a fragment or a derived sequence thereof, said fragment or derived sequence being able to inhibit apoptosis,
- and a nucleotide sequence controlling the level of expression of p53 or bcl-2 proteins, said nucleotide sequence comprising a sequence coding for an activator protein responsible for the expression of p53 or bcl-2 proteins mentioned above either when a specific compound is present in the culture medium, or when a specific compound is absent from the culture medium, the expression p53 or bcl-2 proteins ranging between 0% to 100% depending on the concentration of the specific compound in the culture medium, said specific compound being able to bind to said activator protein thus allowing, or conversely, inhibiting said protein to act as an activator of the promotion of the transcription under the control of which is placed the above-mentioned nucleotide sequence coding for the p53 or bcl-2 protein.

ically transformed cells according to claim 6, characterized in that they are g mammalian neuronal cells such as rat PC12 neuronal cell line or any other lls or cell lines.

enetically transformed cells according to claim 6 or 7, characterized in that the e sequence controlling the level of expression of p53 or bcl-2 proteins, is a Tet- e coding for a tetracycline-controllable transactivator (tTA), the tTA comprising a Tet repressor (TetR) linked to a polypeptide which directly or indirectly activates transcription of p53 or bcl-2 proteins, the transcription of the tTA being under the control of an appropriate promoter such as a promoter from cytomegalovirus (pCMV).

9. Genetically transformed cells according to anyone of claims 6 to 8, characterized in that :

- the polypeptide which directly or indirectly activates transcription of p53 or bcl-2 proteins, is the virion protein 16 from herpes simplex virus (VP16),

- the transcription of the p53 or bcl-2 proteins is under the control of a tTA-responsive promoter comprising a minimal promoter, such as the cytomegalovirus immediate early gene promoter (P_{minCMV}), linked to a tetracycline responsive element (TRE).

10. Process for the detection of developmental and post-developmental toxicity of endogenous or exogenous factors, including drugs and other chemicals, or for the screening of molecules useful as drugs for the treatment of pathologies related to an inhibition or an activation of apoptosis, said process comprising :

- contacting a sample comprising said compounds or molecules to be tested with one or several culture media of genetically transformed cells as defined in anyone of claims 6 to 9, wherein the expression of p53 or bcl-2 protein is from 0% to 100%, each culture medium containing cells expressing a specific proportion of p53 or bcl-2,

- the measure of the cell death threshold in the different cell expressing specific proportion of p53 or bcl-2 culture media, and the comparison of the measured threshold

with the cell death threshold measured in control cells expressing the same specific proportion of p53 or bcl-2 proteins, said control cells having not been contacted with said sample.

11. Process according to claim 10, for the screening of drugs for the treatment of pathologies such as acute and chronic neurological disorders including stroke, and traumatic injuries, ageing, neuroblastomas, Alzheimer's disease, Parkinson's disease, HIV encephalopathy, Prion's disease, Creutzfeld-Jacob diseases, Huntington's disease and several neuromuscular diseases.

12. Kits for carrying out a process according to claim 10 or 11, comprising genetically transformed cells according to anyone of claims 6 to 9, and optionally the specific compound used to regulate the proportion of p53 or bcl-2 proteins in the culture medium, such as tetracycline or doxycycline.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

5

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
WOB 99 AN CEU NEUR		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08223	23/08/2000	27/08/1999
International Patent Classification (IPC) or national classification and IPC C12N15/85		
Applicant EC (EUROPEAN COMMUNITY) et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/01/2001	20.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Roscoe, R Telephone No. +49 89 2399 2554

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/08223

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-22	as originally filed				

Claims, No.:

1-11	as received on	05/11/2001	with letter of	05/11/2001	

Drawings, sheets:

1/4-4/4	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 5, 9-11
	No:	Claims 1-4, 6-8
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-11

Industrial applicability (IA) Yes: Claims 1-11
No: Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

IV. Lack of Unity

Since applicant has paid an additional examination fee, both invention groups have been examined.

The two inventions, both covering claims 1-12(in part), relate to cells transformed with I: construct expressing p53 and II: transformed with construct expressing bcl-2 and uses thereof to assay toxicity. Bcl-2 (XP000876792) has already previously been overexpressed in a system to analyse apoptotic mechanisms, the same applies to p53 (XP000876795). Further, Bcl-2 and p53 have opposite effects and hence the two claimed systems do not solve the same technical problem.

V. Reasoned statement on Novelty, Inventive Step and Industrial Applicability

The documents mentioned in the present written opinion / International Preliminary Examination Report are numbered as in the search report, i.e. D1 corresponds to the first document of the search report etc.

- Novelty (Art.33(2) PCT)

D1 discloses the effect of p53 or of hydrogen peroxide on rat embryo fibroblasts carrying inducible bcl-2 genes. Bcl-2 was shown to slow down at least two stages of apoptosis: decrease of mitochondrial membrane potential and subsequent morphological changes. The bcl-2 gene was cloned downstream of a minimal promoter carrying bacterial tOp sequences. A CMV promoter drives constitutive expression of a tTA fusion protein. In the absence of tetracycline, tTA binds tOp and activates bcl-2 transcription. Addition of tetracycline progressively prevents tTA binding. Cells grown in absence of tetracycline thus express bcl-2 and these cells were challenged with apoptotic inducers and the level of apoptosis determined. Since rat embryo fibroblasts are not neuronal cells, D1 no longer anticipates claims.

D2 discloses a Tet-regulatable His-273 mutant of p53, yet this mutant is not per se capable of inducing apoptosis. Also cotransfected cells with a wild-type p53 expression plasmid (which is assumed to be constitutively expressing p53 since

induction is never mentioned in this context) and fusion peptide 46. Also, tumor cell lines with varying p53 status (wild-type, mutant, null) were treated with peptide 46 and analysed for induction of apoptosis. Nevertheless, active p53 does not appear to have been expressed from inducible system. Hence, not relevant to novelty.

D3 discloses Saos2 (osterosarcoma) and H1299 (small cell lung carcinoma)-derived cell lines having p53 expressed under the control of a tetracycline-regulated promoter. Showed that cells expressing low levels of p53 (levels set by varying level of tetracycline had reduced growth but did not exhibit apoptosis. Further showed that when levels of p53 were low (in tet-regulated cell line which produced lowish levels of p53 even in absence of p53), camptothecin could induce apoptosis in a greater number of cells, despite p53 levels remaining stable. Thus p53 expression could sensibilize cells to induction of apoptosis. It is stated that this situation can be utilized to screen a variety of cancer therapy drugs that may cooperate with p53 to induce apoptosis. Since cells actually employed were not neuronal cells, no basis for novelty objection to present claims.

D4 provides a detailed mechanistic explanation of the Tet system employed (preferably by the applicant). Further, the system is used to express bcl-2 in a NIH/3T3(fibroblast-like)-derived cell line. Further showed that overexpression of Ha-ras from a parallel regulatory system caused apoptosis which could be reduced dramatically by simultaneous overexpression of bcl-2. Since cells actually employed were not neuronal cells, no basis for novelty objection to present claims.

D5 uses a constitutive bcl-2 expressing plasmid to express protein in PC12 cells. Cells were challenged by apoptosis inducers. Not relevant to novelty.

D6 is a review on apoptosis, D7 relates to the regulation of Bcl-2 phosphorylation.

D8 provides a method for evaluating cancer treatments. The p53 gene can be knocked out and the efficacy of the agent to induce apoptosis tested in its absence. D9 disclosure is similar. D10 is irrelevant in present context.

D11 performs various assays on apoptosis in PC12 cells. PC12 is a neuronal cell line which was also used in application. It was shown that bcl-2, which was expressed from a Tet-regulated system, could prevent apoptosis induced by p55-IC. The study is certainly a mechanistic study (compare bottom claim 1). D11 anticipates claims 1-4 and 6-8.

- **Inventive Step (Art.33(3) PCT)**

With respect to the claims in general, it is noted that the functions of bcl-2 and p53 in the context of apoptosis have been recognized and suitable expression systems as used in the above documents are available. Further, it is known that many diseases are associated with the regulation of apoptosis. Hence, the use of the known systems to study such diseases is entirely obvious. D3 even suggests using such a system to screen for anti-cancer drugs. (note: claim 1 refers to neuroblastomas). Obvious that can use system to screen for drugs relating to other disorders associated with apoptosis.

The presently novel claims 5 and 9 are only novel due to involvement of VP16. This is however presently considered an optional and trivial aspect of the regulatory system used.

Claims 10 and 11 are novel since in D11 p55IC is not added to the culture media. It is also arguable whether the process described in D11 can be considered to fall under the kind of screening claimed. Nevertheless, general inventive step objection applies.

- **Industrial Applicability (Art.33(4) PCT)**

The present claims appear to have industrial applicability. Nevertheless it may be better to make it absolutely clear that the cells used in claim 1 cannot be within an animal body.

VIII. **Certain observations**

- **Clarity (Art.6 PCT)**

It is noted that many of the independent claims have features which are optional "such as". These kind of optional features should usually be placed in appended dependent claims.

Claim 11 refers to itself.

CLAIMS

1. Use of mammalian neuronal cells genetically transformed with the following nucleotide sequences :

- a nucleotide sequence coding for the p53 protein or a fragment or a derived sequence thereof, said fragment or derived sequence being able to induce apoptosis,

- or a nucleotide sequence coding for the bcl-2 protein or a fragment or a derived sequence thereof, said fragment or derived sequence being able to inhibit apoptosis,

- and a nucleotide sequence controlling the level of expression of p53 or bcl-2 proteins, said nucleotide sequence comprising a sequence coding for an activator protein responsible for the expression of p53 or bcl-2 proteins mentioned above either when a specific compound is present in the culture medium, or when a specific compound is absent from the culture medium, the expression p53 or bcl-2 proteins ranging between 0% to 100% depending on the concentration of the specific compound in the culture medium, said specific compound being able to bind to said activator protein thus allowing, or conversely, inhibiting said protein to act as an activator of the promotion of the transcription under the control of which is placed the above-mentioned nucleotide sequence coding for the p53 or bcl-2 protein,

for the detection of developmental or post-developmental neurotoxicity of endogenous or exogenous factors, including drugs and other chemicals, or for the screening of drugs for the treatment of pathologies related to acute and chronic neurological disorders including stroke, and traumatic injuries, ageing, neuroblastomas, Alzheimer's disease, Parkinson's disease, HIV encephalopathy, Prion's disease, Creutzfeld-Jacob diseases, Huntington's disease and several neuromuscular diseases, or for mechanistic studies.

2. Use according to claim 1, characterized in that the genetically transformed mammalian neuronal cells are chosen among rat PC12 neuronal cell line or any other eucaryotic neuronal cells or cell lines.

3. Use according to claim 1 or 2, characterized in that the expression of p53 or bcl-2 proteins is proportional to the quantity of the specific compound added to the

culture medium, so that the level of expression of p53 or Bcl-2 in the transformed cells is 0%, or 100%, or comprised between 0% and 100%.

4. Use according to anyone of claims 1 to 3, characterized in that the nucleotide sequence controlling the level of expression of p53 or bcl-2 proteins, is a Tet-Off gene coding for a tetracycline-controllable transactivator (tTA), the tTA comprising a Tet repressor (TetR) linked to a polypeptide which directly or indirectly activates transcription of p53 or Bcl-2 proteins, the transcription of the tTA being under the control of an appropriate promoter such as a promoter from cytomegalovirus (pCMV).

5. Use according to anyone of claims 1 to 4, characterized in that :

- the polypeptide which directly or indirectly activates transcription of p53 or bcl-2 proteins, is the virion protein 16 from herpes simplex virus (VP16),

- the transcription of the p53 or bcl-2 proteins is under the control of a tTA-responsive promoter comprising a minimal promoter, such as the cytomegalovirus immediate early gene promoter (P_{minCMV}), linked to a tetracycline responsive element (TRE).

6. Mammalian neuronal cells genetically transformed with the following nucleotide sequences :

- a nucleotide sequence coding for the p53 protein or a fragment or a derived sequence thereof, said fragment or derived sequence being able to induce apoptosis,

- or a nucleotide sequence coding for the bcl-2 protein or a fragment or a derived sequence thereof, said fragment or derived sequence being able to inhibit apoptosis,

- and a nucleotide sequence controlling the level of expression of p53 or bcl-2 proteins, said nucleotide sequence comprising a sequence coding for an activator protein responsible for the expression of p53 or bcl-2 proteins mentioned above either when a specific compound is present in the culture medium, or when a specific compound is absent from the culture medium, the expression p53 or bcl-2 proteins ranging between 0% to 100% depending on the concentration of the specific compound in the culture medium, said specific compound being able to bind to said activator protein thus allowing, or conversely, inhibiting said protein to act as an activator of the

promotion of the transcription under the control of which is placed the above-mentioned nucleotide sequence coding for the p53 or bcl-2 protein.

7. Genetically transformed mammalian neuronal cells according to claim 6, characterized in that they are chosen among as rat PC12 neuronal cell line or any other eucaryotic neuronal cells or cell lines.

8. Genetically transformed mammalian neuronal cells according to claim 6 or 7, characterized in that the nucleotide sequence controlling the level of expression of p53 or bcl-2 proteins, is a Tet-Off gene coding for a tetracycline-controllable transactivator (tTA), the tTA comprising a Tet repressor (TetR) linked to a polypeptide which directly or indirectly activates transcription of p53 or bcl-2 proteins, the transcription of the tTA being under the control of an appropriate promoter such as a promoter from cytomegalovirus (pCMV).

9. Genetically transformed mammalian neuronal cells according to anyone of claims 6 to 8, characterized in that :

- the polypeptide which directly or indirectly activates transcription of p53 or bcl-2 proteins, is the virion protein 16 from herpes simplex virus (VP16),
- the transcription of the p53 or bcl-2 proteins is under the control of a tTA-responsive promoter comprising a minimal promoter, such as the cytomegalovirus immediate early gene promoter (P_{minCMV}), linked to a tetracycline responsive element (TRE).

10. Process for the detection of developmental and post-developmental toxicity of endogenous or exogenous factors, including drugs and other chemicals, or for the screening of molecules useful as drugs for the treatment of pathologies related to an inhibition or an activation of apoptosis, said process comprising :

- contacting a sample comprising said compounds or molecules to be tested with one or several culture media of genetically transformed mammalian neuronal cells as defined in anyone of claims 6 to 9, wherein the expression of p53 or bcl-2 protein is from 0% to 100%, each culture medium containing cells expressing a specific proportion of p53 or bcl-2,

- the measure of the cell death threshold in the different cell expressing specific proportion of p53 or bcl-2 culture media, and the comparison of the measured threshold with the cell death threshold measured in control cells expressing the same specific proportion of p53 or bcl-2 proteins, said control cells having not been contacted with said sample.

11. Kits for carrying out a process according to claim 10 or 11, comprising genetically transformed mammalian neuronal cells according to anyone of claims 6 to 9, and optionally the specific compound used to regulate the proportion of p53 or bcl-2 proteins in the culture medium, such as tetracycline or doxycycline.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
WOB 99 AN CEU NEUR		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 08223	23/08/2000	27/08/1999
Applicant		
EC (EUROPEAN COMMUNITY)		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
T/EP 00/08223

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/85 C12N5/10 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GUENAL I ET AL: "Bcl-2 and Hsp27 act at different levels to suppress programmed cell death" ONCOGENE, vol. 15, no. 3, 17 July 1997 (1997-07-17), pages 347-360, XP000876792 figures 1-10 ---	1-11
X	SELIVANOVA G ET AL: "Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain" NAT MED, vol. 3, no. 6, June 1997 (1997-06), pages 632-638, XP002130736 figure 3 --- -/--	1-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 February 2001	23/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lonnoy, O

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/EP 00/08223

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CHEN X ET AL: "p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells" GENES & DEVELOPMENT, vol. 10, no. 19, 1 October 1996 (1996-10-01), pages 2438-2451, XP000876795 figures 1-5,8 ---	1-11
X	LIU H ET AL: "Lac/Tet-inducible system functions in mammalian cell lines" BIOTECHNIQUES, vol. 24, no. 4, April 1998 (1998-04), pages 624-632, XP002130737	6,8,9
Y	figure 3 ---	1-5,7, 10-12
Y	SATOH T ET AL: "Free radical-independent protection by nerve growth factor and Bcl-2 of PC12 cells from hydrogen peroxide-triggered apoptosis" J. BIOCHEM., vol. 120, no. 3, September 1996 (1996-09), pages 540-546, XP002130738 cited in the application figures 1,6 ---	1-5,7, 10-12
A	LEIST M ET AL: "Apoptosis, excitotoxicity, and neuropathology" EXP. CELL RES., vol. 239, no. 2, 15 March 1998 (1998-03-15), pages 183-201, XP000876804 the whole document ---	1-5,7, 10-12
A	WO 96 35124 A (UNIV JEFFERSON) 7 November 1996 (1996-11-07) ---	
A	WO 95 05738 A (MASSACHUSETTS INST TECHNOLOGY) 2 March 1995 (1995-03-02) ---	
A	WO 97 24458 A (FOX CHASE CANCER CENTER) 10 July 1997 (1997-07-10) ---	
A	WO 94 26889 A (IST NAZ STUD CURA DEI TUMORI ;GRECO ANGELA (IT); PIEROTTI MARCO A) 24 November 1994 (1994-11-24) ---	

-/--

2

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]T/EP 00/08223

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	HAVIV R ET AL: "The intracellular domain of p55 tumor necrosis factor receptor induces apoptosis which requires different caspases in naive and neuronal PC12 cells" J NEUROSCI RES, vol. 52, no. 4, 15 May 1998 (1998-05-15), pages 380-389, XP000879428	

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
/EP 00/08223

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9635124 A	07-11-1996	US 5695944 A	09-12-1997
		US 6130201 A	10-10-2000
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WO 9724458 A	10-07-1997	US 5741646 A	21-04-1998
WO 9426889 A	24-11-1994	IT 1264447 B	23-09-1996
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		DE 69402313 T	21-08-1997
		DK 698096 T	27-10-1997
		EP 0698096 A	28-02-1996
		ES 2101537 T	01-07-1997
		FI 955447 A	13-11-1995
		GR 3023878 T	30-09-1997
		JP 8510119 T	29-10-1996
		NO 954556 A	12-01-1996
		NZ 267086 A	29-01-1997

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/16304 A2

(51) International Patent Classification[7]: C12N 15/00

(21) International Application Number: PCT/EP00/08223

(22) International Filing Date: 23 August 2000 (23.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
99402139.2 27 August 1999 (27.08.1999) EP

(71) Applicant *(for all designated States except US)*: EC (EUROPEAN COMMUNITY) [LU/LU]; Rue Alcide de Gaspéri, L-2920 Luxembourg (LU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: STINGELE, Silvia [DE/IT]; Via Michelangelo, 6, I-21028 Travedona (IT). COECKE, Sandra [BE/BE]; Dr Persoonslaan 36, B-2830 Willebroek (BE). NICOTERA, Pierluigi [IT/DE]; In den Dorfackern 25, 78465 Konstanz (DE). BALLS, Michael [GB/IT]; Via Brusisch 2587, I-21020 Comabbio (IT).

(74) Agents: DEMACHY, Charles et al.; Grosset-Fournier & Demachy SARL, 20, rue de Maubeuge, F-75009 Paris (FR).

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(54) Title: GENETICALLY ENGINEERED CELL LINES, AND THEIR USES, IN PARTICULAR FOR NEUROTOXICITY TESTING

(57) Abstract: The present invention relates to genetically engineered cell lines, and to their uses, in particular in processes for screening the developmental or post-developmental neurotoxicity of endogenous or exogenous factors, including drugs and other chemicals, also at low-level exposure, for screening pharmaceutically active molecules useful as drugs for the treatment of pathologies involving an abnormal inhibition or activation of cell death, and for mechanistic studies, the said cell lines being genetically transformed with a nucleotide sequence coding for the p53 protein or a nucleotide sequence coding for the bcl-2 protein, and a nucleotide sequence controlling the level of expression of p53 or bcl-2 proteins. The present invention also relates to kits comprising the said genetically engineered cell lines for carrying out the said processes.

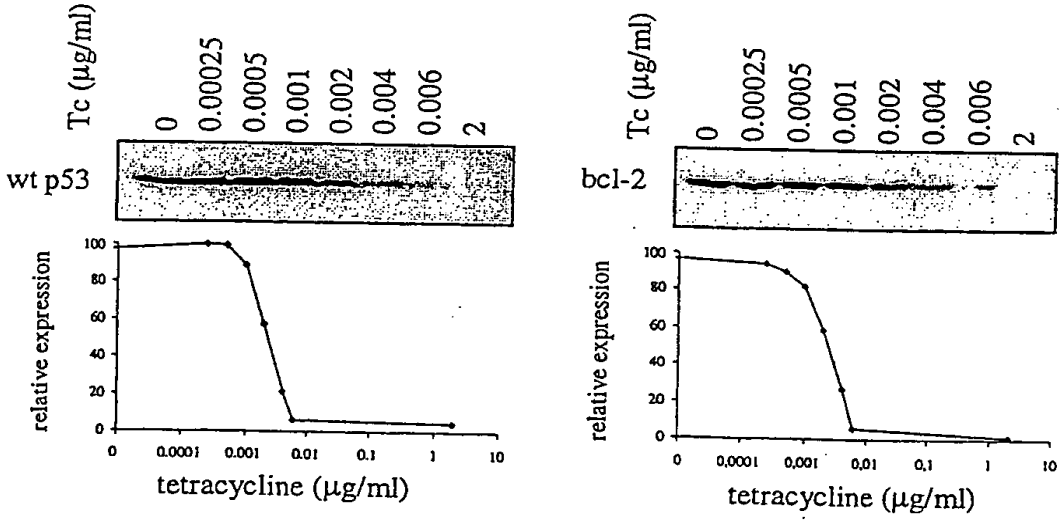
Tc (µg/ml)
0
0.00025
0.0005
0.001
0.002
0.004
0.006
2
wt p53
relative expression
100
80
60
40
20
0
0
0.0001
0.001
0.01
0.1
1
10
tetracycline (µg/ml)
Tc (µg/ml)
0
0.00025
0.0005
0.001
0.002
0.004
0.006
2
bcl-2
relative expression
100
80
60
40
20
0
0
0,0001
0,001
0,01
0,1
1
10
tetracycline (µg/ml)

Figure 1

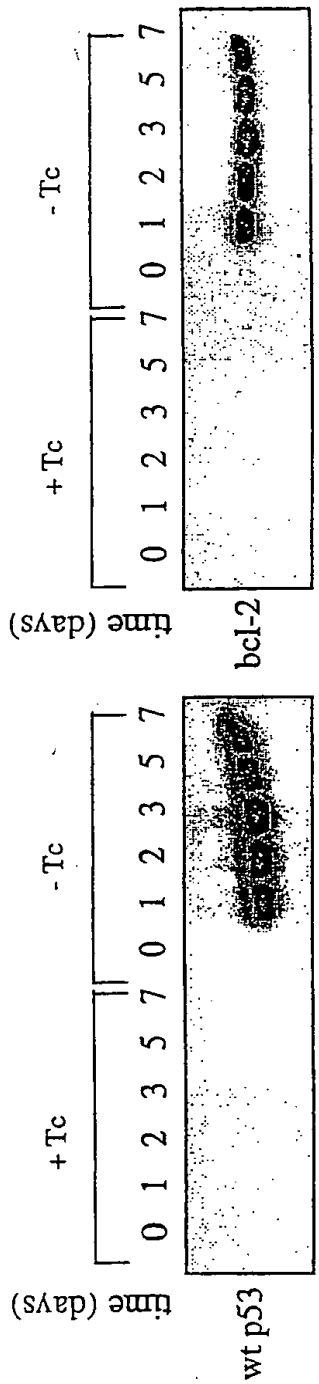
time (days)
+Tc
0 1 2 3 5 7
-Tc
0 1 2 3 5 7
bcl-2
time (days)
+Tc
0 1 2 3 5 7
-Tc
0 1 2 3 5 7
wt p53

Figure 2

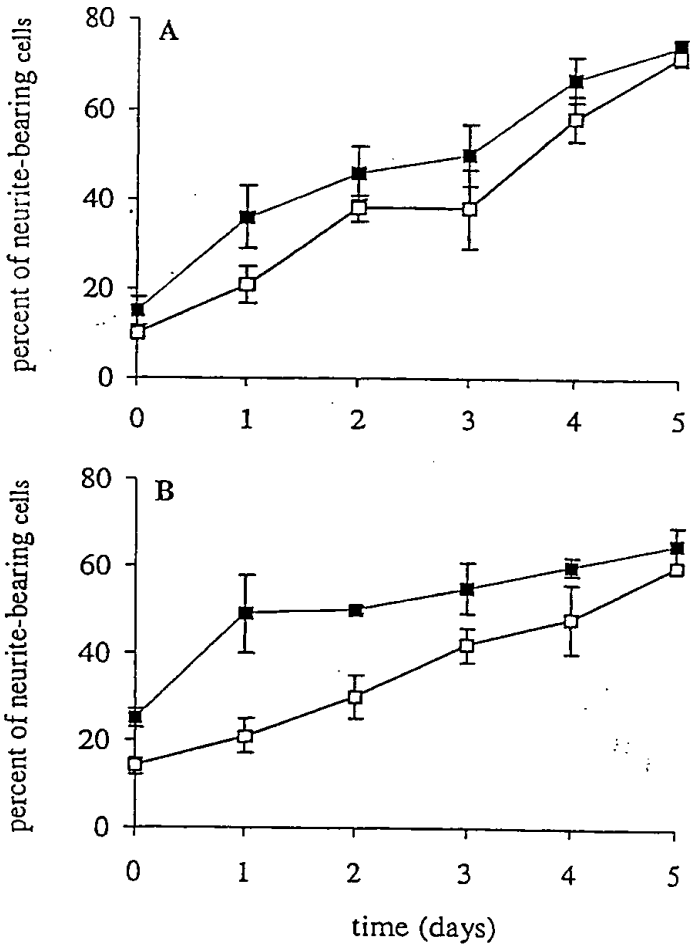
A
B
percent of neurite-bearing cells
percent of neurite-bearing cells
0
20
40
60
80
0
1
2
3
4
5
time (days)

Figure 3

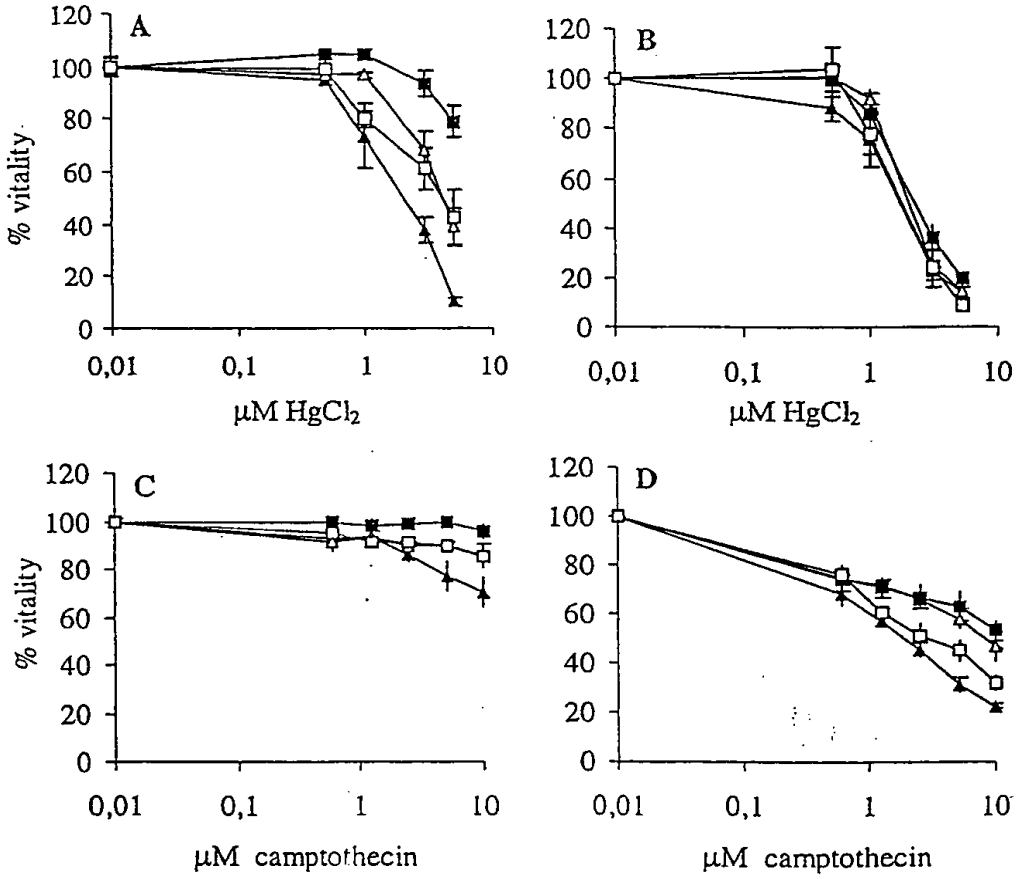
A
B
C
D
120
100
80
60
40
20
0
% vitality
0,01
0,1
1
10
µM HgCl2
µM camptothecin

Figure 4

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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International Bureau

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(51) International Patent Classification[7]: C12N 15/85, 5/10, G01N 33/50

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99402139.2 27 August 1999 (27.08.1999) EP

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/08223

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/85 C12N5/10 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GUENAL I ET AL: "Bcl-2 and Hsp27 act at different levels to suppress programmed cell death" ONCOGENE, vol. 15, no. 3, 17 July 1997 (1997-07-17), pages 347-360, XP000876792 figures 1-10 ---	1-11
X	SELIVANOVA G ET AL: "Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain" NAT MED, vol. 3, no. 6, June 1997 (1997-06), pages 632-638, XP002130736 figure 3 --- -/--	1-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 February 2001	23/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lonnoy, O

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP 8510119 T	29-10-1996
		NO 954556 A	12-01-1996
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Date of the actual completion of the international search	Date of mailing of the international search report
19 February 2001	23/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lonnoy, O

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INTERNATIONAL SEARCH REPORT

tional Application No
PCT/EP 00/08223

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2

INTERNATIONAL SEARCH REPORT

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PCT/EP 00/08223

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2

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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